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<p>19991214 071</p> <p>In some respects, tumors and wounds behave in a similar fashion. Among other things, both require the ingrowth of new blood vessels either to establish healing, or in the case of tumors, to promote tumor growth and metastasis. Our central hypothesis in this study is that tumors express angiogenic factors, and respond abnormally to such factors. In previous work, we established the presence of IL-8, a known angiogenic factor in human breast cancers. This was measured in samples from human tumors, and measured <i>in vitro</i> from human breast cancer cells. This report covers our initial attempts at establishing an <i>in vivo</i> model of human breast cancer, and studies using anti-angiogenesis agents. During the course of this Task, numerous methodological problems were encountered that needed to be resolved. Once these were overcome, we were able to reliably grow the tumor cell lines in immunodeficient mice. Mice were then treated with specific and non-specific inhibitors. Preliminary results demonstrated inhibition of tumor growth by both anti IL-8 antibodies, and thalidomide. This effect was lost at later time points. These studies, and future plans are described. Work is ongoing.</p>			
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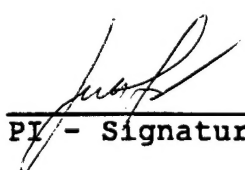
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Annual Report Body

Introduction

Breast cancer and angiogenesis

Breast cancer remains the most common malignancy in women. With the widespread use of mammography, and increased emphasis on early diagnosis, patients are presenting with smaller and lower stage cancers. Unfortunately, despite early diagnosis, tumor cells have metastasized prior to diagnosis and surgical removal of the primary breast tumor. Thus, approximately 1/3 of patients diagnosed with breast cancer will die of their disease. Newer forms and applications of adjuvant chemotherapy are promising. The concept of adjuvant therapy assumes that among the population of women who do not have clinically apparent metastatic cancer, there exists a cohort who already harbor metastases. Patients whose tumors have not metastasized are treated with chemotherapy, but receive no benefit. Sadly, there is no way to distinguish this group of patients from those with metastases that do receive a benefit from this treatment. The frequency of the occurrence of metastases can be predicted with some certainty based on accepted known risk factors. These traditionally have included the size of the primary cancer, the number of involved lymph nodes, and the grade of the tumor. Recently, it has become apparent that tumors depend on the ingrowth of new blood vessels (angiogenesis) for their growth. This process also allows the tumors access to the vascular system, a critical step in the process of metastasis. Little is known about the factors that control this critical process. Angiogenesis is a normal part of injury and repair, and organogenesis, but happens in an inappropriate fashion in cancers. Understanding the factors that control angiogenesis in human cancers will lead to new forms of treatment aimed not only at halting tumor growth, but also directed at preventing metastasis, and arresting the growth of established metastases.

Interleukin 8 and breast cancer

We have examined the cytokines that regulate angiogenesis in wound healing, in order to see if these factors are also found in human cancers. The over-expression of these factors would imply that they might be involved in the regulation of angiogenesis in cancers as well. Our lab is specifically interested in the cytokine interleukin 8 (IL-8). The reason for this choice is well

described in our original application and in previous Annual Reports. This hypothesis led to the formation of the experimental structure of this project. First, we examined specimens from patients to determine whether IL-8 was found in breast cancer tissues. Next, we compared the levels of IL-8 with other known factors associated with poor prognosis such as ER and PR expression. We were able to obtain large numbers of tissue homogenates at low cost by utilizing discarded specimens from ER/PR analysis. Unfortunately, patient information was not available on these samples. As expected, we found that patients whose cancer expressed high levels of IL-8 also had low levels of ER and PR. For that reason, we used ER/PR levels as surrogate markers of outcome. This finding supported our general hypothesis and justified further investigations into the mechanism of this process. Next, we examined the factors that regulated the expression of IL-8 utilizing our *in vitro* models. This work, that has been previously reported in both abstract and manuscript demonstrated that breast cancer cells produce IL-8 *in vitro* and described the regulatory factors involved. We found that IL-1 and TNF play an important role in the production of IL-8 by the tumor cells. As a result of these observations, we then went back to our human material to see if there were receptors on the tumor cells for these regulatory cytokines. In order to support our hypothesis that tumor cells were producing IL-8, it would be critical to demonstrate such receptors on the tumor cell surfaces. Since we hypothesize that IL-8 is playing an important role in angiogenesis, we also need to demonstrate that there are receptors for IL-8 on blood vessels associated with tumors. Similarly, we believe that IL-8 is also acting in an autocrine fashion, stimulating the tumor cells as well as the associated blood vessels. We have not excluded the possibility that infiltrating cells such as macrophages and leukocytes, normally recruited to injuries could also be contributing to this process. Our immunohistochemical studies clearly demonstrated the presence of these receptors both on tumor cells and associated blood vessels.

IL-1 Family and Cancer

Cytokines have been implicated as important regulators of cell function in a variety of diseases. For example, cytokines have been considered to be key regulators of tissue cells and leukocytes in chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and interstitial lung disease to name but a few. It has been further postulated that in the

case of acute and chronic inflammation, cytokines such as IL-1 and TNF are present in the tissue microenvironment in quantities sufficient to control both the inflammatory and repair processes. Thus, the important role of cytokines in general, and IL-1 specifically, in inflammation and wound healing is clearly established. The foundations of our hypothesis on the role of IL-1 in human breast cancer are presented below.

Currently the IL-1 family of cytokines and receptors encompasses two agonist polypeptides (IL-1a and IL-1B), a competitive antagonist (IL-1RA) and two receptors (IL-1RI and IL-1RII). Interleukin-1 (IL-1) designates two distinct proteins, IL-1a and IL-1B. These forms are structurally related and show approximately 25% amino acid homology. IL-1a is the acidic form while IL-1B is the neutral form. Both IL-1a and IL-1B are synthesized as 31kD precursors, which are cleaved into 17kD proteins. Interestingly, these cytokines lack classical signal peptides (for secretion) yet while IL-1a remains intracellular; IL-1B is secreted out of the cell. IL-1a and IL-1B exert their physiological effects by binding to specific receptors. There are two IL-1 receptors, IL-1 receptor I (IL-1RI) and IL-1 receptor II (IL-1RII). IL-1RI is a 80kD membrane bound receptor while IL-1RII is 68kD's. They are both members of the Ig superfamily. The two receptors share 28% homology in their extracellular domains but differ in their cytoplasmic regions. Where IL-1RI has a 213 amino acid cytoplasmic domain, IL-1RII contains only 29 amino acids in this region. IL-1RI is the signal transducing receptor and IL-1RII does not transduce signal when IL-1 is bound to it. Generally, IL-1a binds with higher affinity to IL-1RI while IL-1B binds better to IL-1RII. IL-1RI is found on endothelial cells, hepatocytes and T lymphocytes while IL-1RII can be found on B lymphocytes, monocytes and neutrophils.

In vitro studies have demonstrated that IL-1 activity can be inhibited by the naturally occurring receptor antagonist designated IL-1 receptor antagonist (IL-1RA). These studies have further demonstrated that the ratio of IL-1RA to IL-1 in the range of 100:1 to 1000:1 can inhibit IL-1 activity. Investigators have studied the role of IL-1 and IL-1RA in wound healing, sepsis and chronic inflammatory diseases such as arthritis and inflammatory bowel disease. In these studies, a decrease in the ratio of IL-1RA to IL-1 was associated with a more severe disease state (i.e. more inflammation and tissue destruction).

A considerable volume of literature exists demonstrating the in vitro ability of IL-1 to regulate a variety of cellular functions in tumor cells. Interestingly, IL-1 has a diverse and

sometimes contradictory effect on the functions and growth of tumor cells. For example, in some instances IL-1 exerts growth-inhibitory activity, yet in other situations IL-1 stimulates tumor growth. In the case of breast cancer, In vitro studies have shown that IL-1 can induce a variety of factors and functions in breast cancer cells. For example, malignant human breast tumors are known to contain high levels of prostaglandins. In a recent study, IL-1B induced PGE2 production in breast fibroblasts. In that study, only two BCC lines, MDA-MB-231 and Hs578T demonstrated increases of PGE2 in response to IL-1B. Several other BCC lines did not respond. IL-1 is also known to inhibit the growth of cultured BCC. For example, insulin and insulin like growth factor I (IGF-I) induced BCC proliferation was inhibited by the presence of IL-1a and IL-1B in MCF-7 cells. In that study, insulin receptor protein and mRNA were increased in the presence of IL-1B. Additional data in that study suggests that IL-1 antagonizes insulin and IGF-I mitogenic effects in MCF-7 by blocking tyrosine kinase. Danforth et al, examined the ability of IL-1 and IL-6 to inhibit BCC growth in vitro. These investigators found that both IL-1 and IL-6 inhibited growth of MCF-7 BCC. IL-1 alone had a greater effect than IL-6 alone. When the two were combined, the effect was synergistic. Further, IL-1 and IL-6 decreased the estradiol stimulated growth of the BCC. Additionally, their studies also demonstrated that IL-1, but not IL-6, caused increased secretion of TGF-b by the BCC. Interestingly, studies by Speiser et al found that IL-1 up-regulated HLA class I and HLA Class II (DR) antigen expression on the cell surface of ZR-75-1 cells. This up-regulation of antigens was associated with increased TNF expression. Thus, IL-1 appears to play an important distribution in the regulation of breast cancer cell function and growth in vitro. Surprisingly little is known about the presence, distribution and role of IL-1 in human breast cancer in vivo, and nothing is know about IL-1 receptors in cancer. Our present study is intended to fill this gap in our knowledge regarding the presence and localization of IL-1 receptors in human breast cancer.

A recent study by Oelmann et al found that glioblastoma cell lines over expressed IL-1RA. Few studies have examined the role of IL-1RA in solid tumors. Expression of IL-1RA has been reported in endometrial cancer, bronchogenic carcinoma, and head & neck squamous cell carcinoma. In the studies by Van Le and Smith increased levels of IL-1RA was found in tumor cells when compared with normal tissue. In the study by von Biberstein a lower but not significant, level of IL-1RA was seen in the tumor versus normal tissue. Interestingly, while

some believe that IL-1RA has the role of blocking IL-1 induced inflammation and disease, others have hypothesized that IL-1RA actually can support the malignant growth by blocking the growth-inhibiting autocrine loop of IL-1. Needless to say, this conflicting data indicates the complexity of the network of IL-1, its receptors, and its antagonist and the need for further study in this area. Our present study revealed the expression of IL-1RA by all of the malignant and non-malignant ductal epithelial cells. Analysis of the breast tumor homogenates tested showed that higher IL-1RA levels were indicative of higher ER levels, or a marker for better outcome. To gain a better understanding of the complex interactions between IL-1 and IL-1RA and the effects the relative levels of these cytokines have in breast cancer, we examined the ratio of IL-1RA to IL-1 in each patient and analyzed the results.

Few studies have investigated the *in vivo* relationship of IL-1 and IL-1RA in human cancer. von Biberstein et al examined the ratios of IL-1RA to IL-1 in head and neck tumor homogenates and found, as in the inflammatory diseases, that the ratio of IL-1RA to IL-1 was significantly lower in the tumor tissue as when compared to the control group. Based on past studies of inflammatory diseases and the current data regarding head & neck cancer it has been suggested that the local balance of IL-1RA to IL-1 may influence tumor growth.

Since IL-1 is a known inducer of IL-8 we have also begun to investigate the potential role in IL-8 expression in HBC. Additionally, using immunohistochemical techniques we demonstrated IL-1 α and IL-1 β are expressed by HBC tumor cells in HBC tumor tissue. Our immunohistochemical studies also successfully demonstrated the presence of IL-1 α and IL-1 β receptors on the tumor cells. Thus, in summary, the work done in Years 01-03 and completed in Year 04 demonstrated that IL-8 was present in human breast cancer, was associated with tumor cells and blood vessels, and correlated with markers of poor outcome. Further receptors for IL-8 and for the cytokines involved in the regulation of IL-8 expression were also present in the tumor microenvironment.

Xenograft model of breast cancer

As a result of these observations, we next sought to examine the expression of IL-8 in a xenograft model of HBC. This portion of the project is the subject of this Annual Report. We encountered a number of unexpected obstacles in the construction of this model. These obstacles

and our approach and ultimate resolution are described. Due to the amount of time involved in solving these problems, only limited new data was obtained. Fortunately, we were able to conserve much of the funds needed until the technical details were resolved. With the extension without additional funds, we have been able to continue this portion of the project. The final results will be completed and submitted with the Final Report.

SPECIFIC AIM III- TO CHARACTERIZE IL-8 ANTIGEN EXPRESSION AND NEOVASCULARIZATION IN HUMAN BREAST CANCER CELLS GROWN AS TUMORS IN NUDE MICE

Study IIIA: To demonstrate IL-8 antigen expression in subcutaneously implanted human breast cancers

Introduction and Rationale: Our original hypothesis was that IL-8 is an important angiogenic factor regulating neovascularization and tumor growth in human breast cancer. Our earlier *in vitro* and immunohistochemical studies of human breast tissue clearly demonstrated that human breast cancer cells are capable of producing IL-8, and that IL-8 is found in association with breast cancer cells in human tumors. Based on these observations, we next sought to establish the presence and distribution of IL-8 in an *in vivo* model of human breast cancer in immunodeficient mice. This step was fundamental to our planned experiments to manipulate the expression of angiogenic factors in the mice in order to control tumor growth.

Obstacles: In order to study the expression and growth of tumors *in vivo*, we selected tumor cell lines based on our *in vitro* data, with high (MDA-MB-231), medium (ZR-75) and low (MCF-7) IL-8 expression. Our early attempts at growing breast tumor cell lines in immunodeficient mice demonstrated highly variable success rates, usually less than 50% success in tumor growth. We reviewed the literature exhaustively and called several prominent investigators working in the area of human mouse xenografts. Through these efforts we discovered that this low success rate for human –mouse xenografts was not unusual. However, we felt that improvements could be made to better guarantee obtaining consistent tumor growth for our studies using angiogenesis inhibitors (see below). To that end we have adapted the techniques used by other laboratories to enhance tumor growth and reproducibility in the immunodeficient mice. Specifically, we have incorporated Matrigel® into the tumor cell- media solutions used for injection of the tumor cells into the mice. The use of this matrix increased our yield of tumor growth to almost 100%. Matrigel is the tumor matrix derived from mouse tumor cells grown *in vitro*, and is known to promote tumor growth *in vitro* due to the presence of both basement membrane, and a large

number of tumor growth factors. Thus we hypothesize that the Matrigel provides a matrix that allow the tumor cells to initially adhere as well as provide numerous growth factors all of which provide a favorable environment for growth of the tumors in the early stage. Overcoming this obstacle significantly delayed the completion of this project but it was essential to develop a reliable tumor model for our inhibitor studies. Once this was overcome, the Studies outlined could proceed.

Results: Immunohistochemical analysis of the xenograft human breast cancer tumors indicated that all three tumor cell lines expressed IL-8 antigen when grown *in vivo*. Additionally, immunohistochemical analysis of these same tumors for IL-8 receptor also indicated that they expressed these receptors *in vivo*. Interestingly we saw that there was a differential expression of the IL-8 receptors on the various HBC tumor cells (see table 1). These observations are consistent with our previous *in vitro* and human breast cancer specimen observations.

TABLE 1: SUMMARY OF TUMOR ASSOCIATED IL-8/IL-8R IMMUNOHISTOCHEMICAL STAIN PROFILES IN HBC-MOUSE XENOGRAFT TISSUE

HBC CELL LINES	IL-8	IL-8 RA	IL-8 RB
MDA-MB-231	+++	-	+
ZR-75	++++	+	+/-
MCF-7	+	-	+/-

1+=faint; 2+=definite; 3+=moderate; 4+=strong

We then analyzed homogenates of the explanted xenograft tumors for IL-8 levels by ELISA. The results of this analysis confirmed the expression of IL-8 by breast tumors *in vivo*. The values obtained paralleled our observations from our *in vitro* experiments described in previous reports, i.e. high IL-8 producers *in vitro* were also high IL-8 expressers *in vivo*, and low producers *in vitro* tended to be low producers *in vivo*. Currently there are no quantitative ELISA analyses available for the IL-8 receptors; thus quantitation of IL-8 receptors could not be done.

TABLE 2: IL-8 EXPRESSION IN XENOGRAFT MODEL COMPARED TO IL-8 EXPRESSION IN *IN VITRO* CELL CULTURE

HBC CELL LINES	IL-8 levels in vitro (pg/ml (IL-1 β stimulation))	IL-8 levels In vivo (pg/mg protein)
MDA-MB231(high IL-8 expresser)	356,960 \pm 61100	34.0; 9.3; 16.0 (n=3)
ZR-75 (mod.IL-8-expressors)	32,600 \pm 4080	N.D.
MCF-7 (low IL-8 expresser)	40 \pm 13	10.4; not detectable (n=2)

Interpretation: The limited number of mice included in this study is a reflection of the difficulty we encountered in setting up this model. However, the *in vivo* results parallel the previous *in vitro* data we reported. The tumor cells maintain their ability to express IL-8 when grown in immunodeficient mice. The cell lines that were high and moderate expressers of IL-8 *in vitro* were higher expressers of IL-8 *in vivo*. These observations support the use of this model in later experiments designed to manipulate angiogenic factors.

Future Plans: This experiment formed the foundation for the studies described below. Our approach was to 1) specifically block IL-8 with monoclonal antibodies, and 2) non-specifically inhibit angiogenesis using a known angiogenesis inhibitor (see below).

Study IIIB: To establish a dual tumor model using MCF-7 and BT-20 cells

Our success in growing the tumors, and identification of IL-8 in the mouse tumors led us to experiments involving anti-angiogenic agents. For that reason it was decided to pursue those studies and postpone or eliminate this study.

Study IIIC: The role of angiogenesis inhibitors

Experiment 1: Tumor growth rates

Introduction and rationale: In order to study the effects of angiogenesis inhibitors on implanted tumors, we first needed to establish the baseline rates of growth of the tumors under standard conditions. We selected the same cell lines used in the previous study. Our approach was to characterize the growth of both a high and low expressers of IL-8 in the xenograft model.

If our hypothesis was correct, that IL-8 is an important factor in tumor growth and angiogenesis, then tumor growth by AF blockers or inhibitors, anti IL-8 or thalidomide, would be greatest in the IL-8 producing tumors. Further, the relative inhibition of tumor growth would be greatest in the tumors that expressed the highest level of IL-8.

Obstacles: Similar difficulties were encountered in these experiments that were conducted simultaneously with studies IIIa. Having established the optimal conditions for growing HBC cell lines in immunodeficient mice, allowed us to obtain the preliminary data presented.

Methods: The same cell lines used for tumor growth kinetic studies were used in this experiment. Briefly, 1×10^7 tumor cells were suspended in Matrigel and injected into the mammary pads of the mice.

Results: Our growth studies indicated that HBC cell lines that were high IL-8 expressers grew more rapidly than did the moderate or low IL-8 expressing strains in our xenograft model (figure 1).

Interpretation: This data demonstrates that there is variation in tumor growth in the xenograft model and that the growth correlates with the general levels of IL-8 expression in vitro and in vivo, supports our hypothesis on the role of tumor cell derived IL-8 in HBC growth.

Future Plans: The number of vessels identified in the tumors will be counted. This will allow us to compare the rate of angiogenesis in the tumors tested. If our hypothesis regarding IL-8 is correct, higher levels of angiogenesis will be found in the tumors from HBC that are high expressers of IL-8.

Experiment 2: Anti-IL-8 antibody injection

Introduction and rationale: Our central hypothesis is that IL-8 is an important AF and growth factor for human tumors. The experiments described above established that HBC express IL-8 *in vivo*, and that the tumors grown in immunodeficient mice express receptors for IL-8. The next phase of this study was to determine if the addition of anti-IL-8 antibody to the mice would inhibit tumor growth.

Methods: The same cell lines used for tumor growth kinetic studies were used in this experiment. Briefly, 1×10^7 tumor cells were suspended in Matrigel and injected into the mammary pads of the mice. Two injection sites were used per mouse. Anti IL-8 antibody was prepared as previously described. The antibody was prepared in PBS at a concentration of 10

mg/ml. Beginning on Day -1, relative to HBC cell line injection, 0.5 ml of antibody was injected i.p into the mice. Control IgG was prepared and injected i.p. at the same schedule as the specific antibody. Tumor growth was measured using calipers twice a week. After 5 weeks, the mice were sacrificed, and the tumor harvested. The tumors were cleared of surrounding tissue and weighed.

Obstacles: Significant problems were encountered in repeated i.p. injections in the mice two times a week. On sacrifice of the mice, multiple adhesions, and evidence of peritonitis and adhesions/fibrosis was seen. The inflammation/adhesions/fibrosis, seen in response to the repeated i.p. injections, likely resulted in erratic absorption of the antibody from the peritoneal cavity. We also postulate that after repeated injections, immune complexes may develop at tumor sites that could mediate macrophage/PMN mediated ADCC reactions. These obstacles can easily account for the results described below.

Results: Tumor growth is plotted as size in mm² in Figure 2A and 2B. Early results seemed to indicate inhibition of tumor growth when compared to the control IgG injected mice. Unfortunately, these results did not persist. Additionally the decrease in tumor size was likely to the extensive necrosis that occurred in the later stages of tumor growth.

Interpretation: The early inhibition of tumor growth appeared quite promising. The anti IL-8 Ab may have been effectively blocking tumor growth in the early stage. Our observation regarding the development of adhesions and inflammation in the peritoneum of the mice can also account for the variability in tumor growth seen. As time progressed, the number of IL-8 receptors may have increased, or the amount of IL-8 produced by the tumor cells may have increased. The net result would be a need for increasing doses of anti IL-8 antibody.

Future Plans: For the above-mentioned reasons, it became evident that theoretic as well as practical considerations dictated that we not continue these studies at this time. Instead attention was paid to a non-specific inhibitor of tumor angiogenesis, thalidomide.

Experiment 3: The role of thalidomide on tumor growth

Introduction and rationale: Despite the difficulties encountered on Experiment 2, the rationale for inhibiting tumor growth by blocking angiogenesis remains valid. Thalidomide has been shown to inhibit angiogenesis when given orally inflammatory models of neovascularization.

However in our xenograft model, gavage feeding is impractical. Likewise, simple addition of thalidomide to the drinking water of the mice would have resulted in unpredictable dosing, due to multiple animal housing and spillage. For these reasons, we elected to prepare the drug in absorbable pellets that could be implanted subcutaneously. The rationale was that this approach would allow for consistent, accurate drug release.

Obstacles: Technology for consistent delivery of thalidomide has not been developed thus we had to develop it. Thus we utilized pellets to give sustained drug release. Short-term high burst of thalidomide possibly accounted for early encouraging results, but with time local drug levels were lost. Clearly a more sustained drug release system will need to be developed

Results: Encouraging early results, suggest that thalidomide may be a potential useful inhibitor of angiogenesis, but a more sustained drug delivery system must be developed.

Future Plans: in future studies we hope to use PEG biodegradable beads as a better thalidomide delivery system to test thalidomide's usefulness as an anti angiogenic drug.

Specific Aim IV-Additional Studies

Study IVA: Interleukin 1 family and Breast Cancer

Previous studies have demonstrated the key role of the IL-1 family of cytokines and receptors in a wide number of immunologic and inflammatory diseases, but little is known about the existence and role of these cytokines and their receptors in human cancers, including human breast cancer. The importance of IL-1 family of cytokines and receptors in disease has lead us to develop the following hypothesis: 1) Human breast tumor cells express the IL-1 family of cytokines (IL-1a, IL-1B and IL-1RA) and receptors (IL-1RI and IL-1RII) and; 2) the local expression of the IL-1 family of cytokines and receptors within the tumor microenvironment can control tumor expression of protumorigenic cytokines such as IL-8. To begin to test this hypothesis we characterized the in vivo and in vitro expression of IL-1a, IL-1B, IL-1RA as well as the IL-1 receptors RI and RII by human breast cancer cells. To initially demonstrate the presence and the distribution of IL-1 cytokines and receptors, as well as IL-8 in human breast disease, archival specimens from 7 benign, 8 DCIS, and 25 invasive human breast tumors were analyzed using standard immunohistochemical techniques. Immunohistochemical studies demonstrated that IL-1a, IL-1B, IL-1RA as well as IL-1RI and IL-1 RII were expressed on both DCIS and invasive

tumor cells. Additionally, IL-1RI receptors appear to be expressed at higher levels in invasive breast tumor cells when compared to DCIS tumor cells and benign breast disease. Interestingly, vascular endothelial cells, fibroblasts and smooth muscle cells in the tumor microenvironment also expressed IL-1 receptors. We next determined the HBC tissue levels and correlation's of the IL-1 and IL-8 cytokines, using ELISA technology. Quantitative studies of the tumor homogenates demonstrated that not only IL-1a and IL-1B were present in the tumor tissue, but that IL-8 was also present. Additionally we demonstrated that IL-1a and IL-1B levels correlated directly with IL-8 levels in HBC tissue. Parallel studies using these same immunoassays on tumor tissue from tumor lines (MCF 7, ZR 75, and MDA) grown in a nude mouse xenograft model of HBC, indicated that the tumor cells also expressed the IL-1 family of cytokines and receptors and IL-8 in vivo. To directly demonstrate the ability of these HBC cell lines to express IL-1 and IL-8 cytokines we initiated in vitro studies. Our in vitro studies demonstrated that human breast cancer cell lines (MCF 7, ZR 75 and MDA) not only expressed IL-1a, IL-1B IL-1RA, IL-1RI and IL-1 RII, but that these tumors cells can be induced by IL-1a or IL-1B to express the protumorigenic cytokine IL-8. These data clearly demonstrate the presence and distribution of IL-1 cytokines and receptors in HBC, and suggest that the local expression of IL-1 by tumor cells likely results in the activation of a number of cells in the tumor microenvironment, productive of the expression of numerous protumorigenic activities such as IL-8 which would induce angiogenesis, tumor proliferation, and tumor invasion. These studies also suggest that targeting of the IL-1RI receptor may provide new approaches in HBC therapy.

Summary

Work presented in previous reports, and published by us established the presence and activity of IL-8 in human breast cancer. The focus of Task 3 was to extend these observations in an in vivo model in immunodeficient mice. Establishing the model for this project proved more difficult than we anticipated. A considerable effort was needed to bring the model to a point where meaningful experiments could be conducted. We finally established a protocol that resulted in fairly consistent growth of tumors in the mice.

Having established the model, we proceeded with two experiments aimed at studying the

effects of both a specific (anti IL-8Ab) and non-specific (thalidomide) inhibitor of angiogenesis. Once again, considerable methodological problems needed to be worked out. Although early results with both inhibitors were promising, sustained responses were not observed. This may reflect methodological issues, or biologic factors. The remainder of our efforts on this project will be directed at sorting out this issue. As we approach the final report on this project.

FIGURE 1

GROWTH CURVES FOR AB CONTROLS FOR THREE CELL LINES

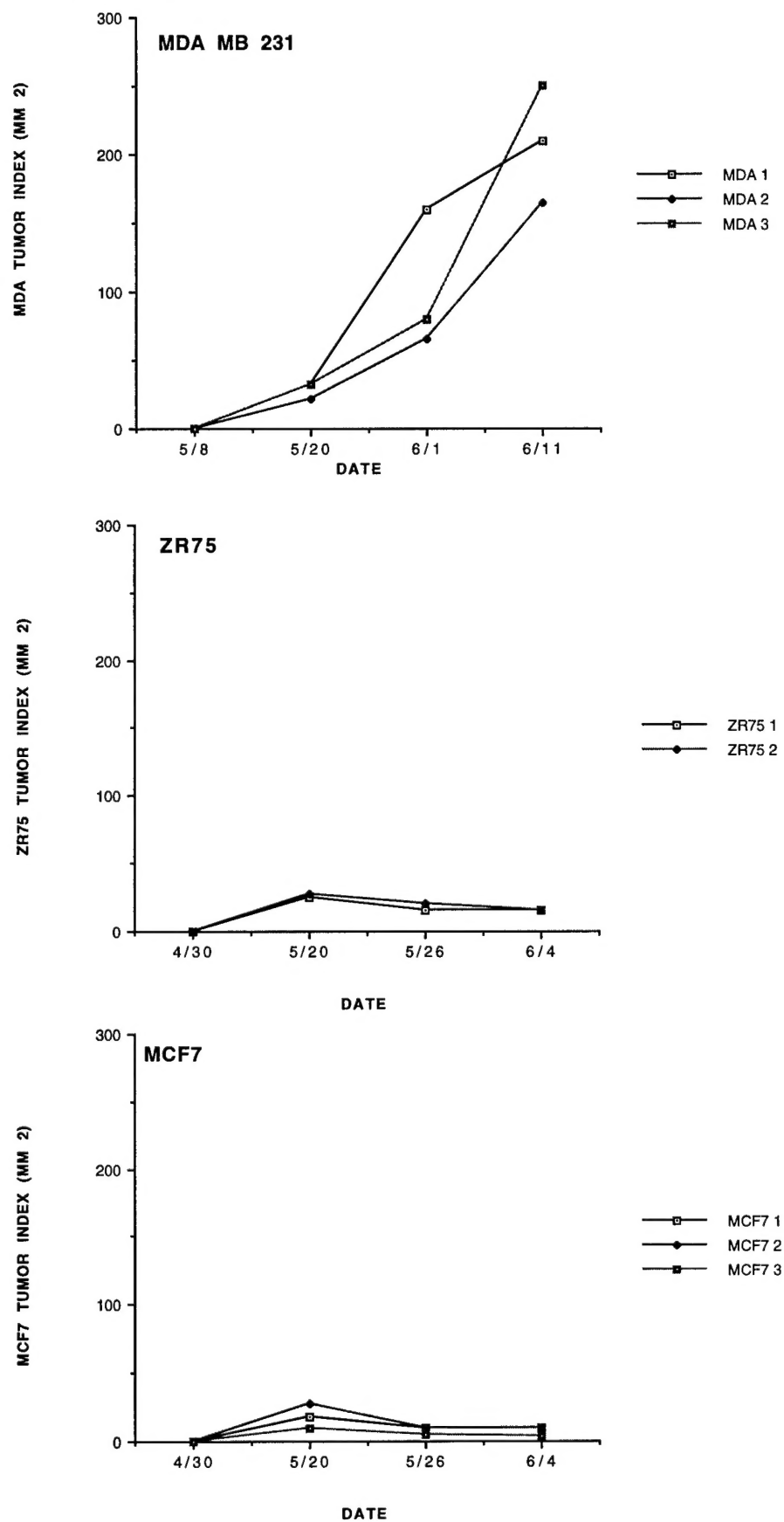
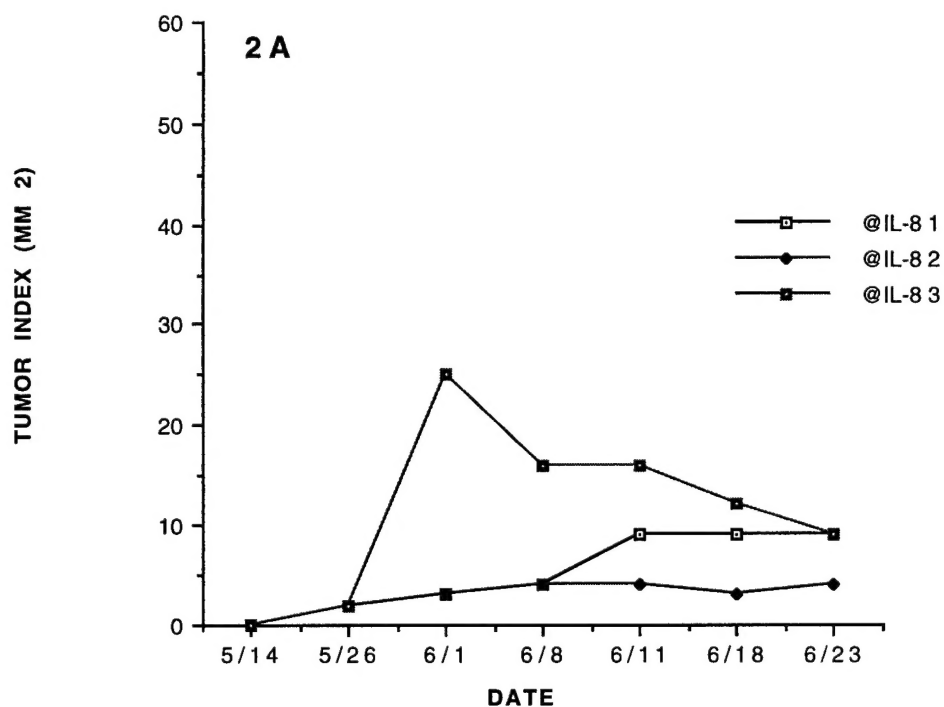
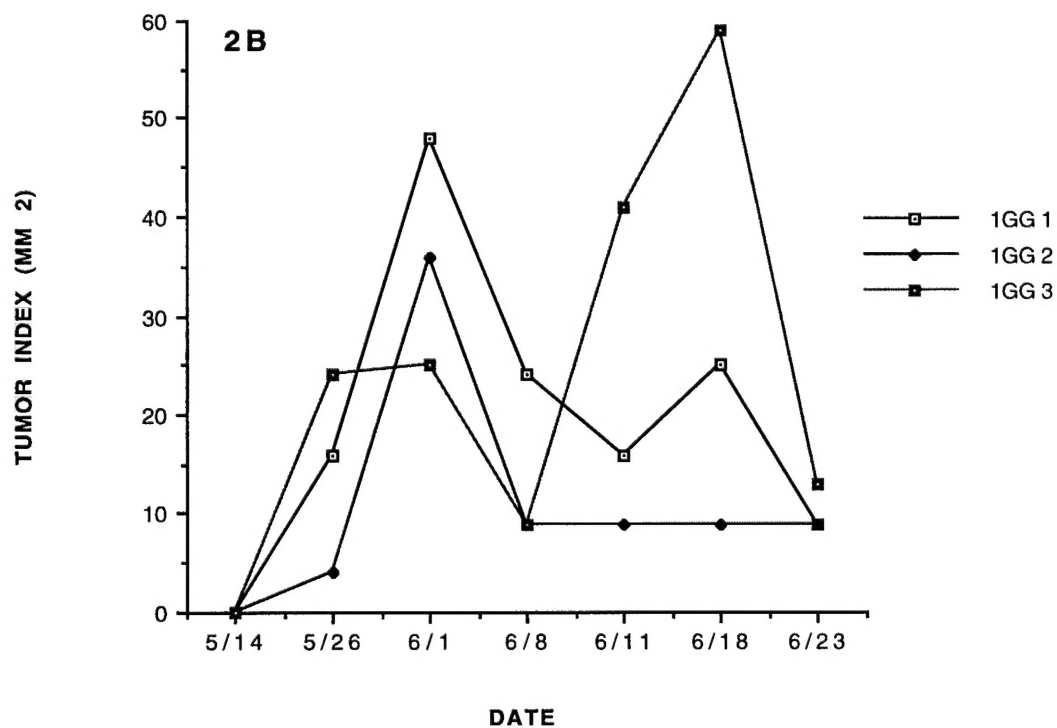


FIGURE 2

ZR-75 TUMOR GROWTH IN @IL-8 INJECTED MICE



ZR-75 TUMOR GROWTH IN IGG INJECTED MICE



Original Statement of Work

Task 1 Year 1 - To characterize IL-8 expression in human breast cancer

Study IA- To characterize IL-8 antigen distribution in human breast biopsy specimens

Study IB- To demonstrate IL-8 mRNA expression in human breast cancer tissues

Study IC- To Correlate IL-8 expression with neovascularization in human breast biopsy specimens

Task 2 Year 2- To characterize IL-8 expression by breast cancer cell lines in vitro

Study A- To characterize the expression of IL-8 antigen by cultured human breast cancer cell lines

Study B- To quantify IL-8 expression in cytokine stimulated breast cancer cells

Study C- To quantify IL-B expression in co cultures of MCF-7 and BT-20 cells

Task 3. Years 3-4- To characterize IL-8 antigen expression and neovascularization in human breast cancer cells grown as tumors in nude mice

Study A- To demonstrate IL-8 antigen expression in subcutaneously implanted human breast cancers

Study B- To establish a dual tumor model using MCF-7 and BT-20 cells

This Statement of Work has been modified to reflect 75% of the original budget. The final study has been omitted due to the lack of resources. Based on results from Year 03, the *in vivo* portion of this study will likely be modified